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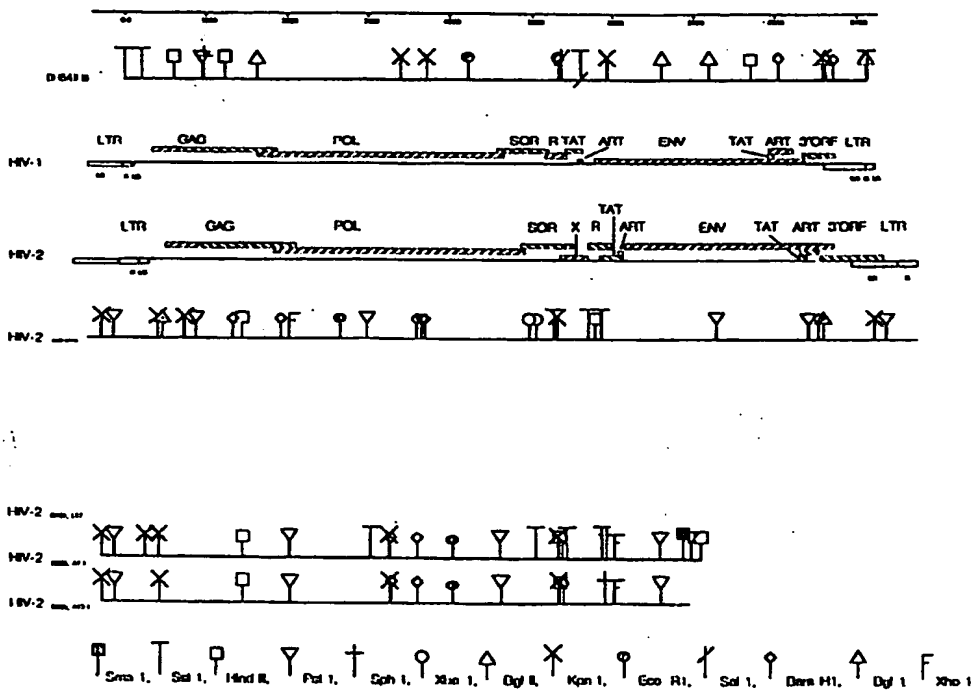
## (54) HIV-2 virus variants.

(57) HIV-2 virus variants, namely virus HIV D205, which can be cloned from the corresponding virus isolate HIV D205 (ECACC V 87122304) and its RNA or RNA-fragments and DNA and DNA-fragments derived therefrom and/or proteins and the use thereof for diagnostics and therapy.

EP 0 655 501 A1


LTR
GAG
POL
SOR
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3'ORF
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HIV-1
LTR
GAG
POL
SOR
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ENV
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ART
3'ORF
LTR
HIV-2
HIV-2
HIV-2
HIV-2
HIV-2
Sma I.
Sal I.
Hind III.
Pst I.
Sph I.
Xba I.
Dgl II.
Kpn I.
Eco RI.
Sal I.
Bam HI.
Dgl I
Xho I

Fig. 1

The present invention relates to HIV D205 a HIV-2 virus variant that may be cloned from the corresponding virus isolate HIV D205 (ECACC V 87122304).

"Molecular cloning of two West African human immunodeficiency virus type 2 isolates which replicate well on macrophages: a Gambian isolate from a case of neurologic aquired immunodeficiency syndrome, and a highly divergent Ghanesian isolate" (Kühnel, H., v. Briesen, H., Dietrich, U., Adamski, M., Mix, D., Biesert, L. Kreutz, R., Immelmann, A., Henco, K., Meichsner, Ch., Andreesen, R., Gelderblom, H. & Rübsamen-Waigmann, H., 1989, Proc. Natl. Acad. Sci. 86, 4, 2383-2387.

In diagnostics, two criteria are demanded to be met, namely specifity and sensitivity for the antigen to be detected. In the diagnostics of AIDS the demand for specifity can certainly be complied with by using the isolates HTLV-$III_B$ and LAV-2 (Guyader, M. et al., "Nature" 326, 1987, 662-669) in order to delimit HIV infections from other infections and, thus, to make a rough assignment into the classes of "HIV-2-related infections" or "HIV-1-related infections". However, a problem is constituted by the sensitivity of the diagnosis. In the range of the so-called seroconversion, i.e. the initial occurrence of the antibody in the infected person, a reduction in sensitivity implies an increase in the number of "falsely negative" test results. Accordingly, it is one main goal to shorten the period between an infection and the detectability of this infection as much as possible by improving the test sensitivity.

A decreased cross reactivity, in the practice of the widely employed ELISA diagnostics, is manifested, for example, in a reduced sensitivity. Thus, the use of the described HIV-1 isolate means about an average reduction of the test sensitivity against HIV-2 sera by the factor of 100 to 1000, whereas the isolate HTLV-$III_B$ enables almost no detection to be accomplished anymore.

A disastrous principle of the diseases caused by HIV resides in the fact that there is not only one type of each of HIV-1 and HIV-2 virus phenotypes and genotypes. What is to be premised is rather a large group of related viruses, possible even populations which by no way are strictly separated from each other but continuously penetrate one another and undergo some evolutionary development to a more and more increasing divergence, while at the same time they begin by recombination events to exchange between each other parts of the genom. Thus, the existing HIV species form a broad continuous population level in which there are no narrowly delimited subpopulations of one virus variant. There is rather to presumed that a continuum exists which is subject to permanent fluctuations with time.

The classified virus variants HIV-1 and HIV-2 are representatives of the diffusely delimited sub-populations having a relative low degree of relationship, which is manifested by only a partial cross reactivity. On the other hand, there are variants of the HIV-1 group (Rübsamen-Waigmann, H. et al., "AIDS-Forschung" 10, 1987, 572-575; Rübsamen-Waigmann, H. et al., J. Med. Virol. 19, 1986, 335-344; v. Briesen, H. et al., J. Med. Virol. 23, 1987 51-66), which do significantly stronger cross-react with HIV-2 than the first characterized HIV-1 isolate itself (Hahn, B. et al., "Nature" 312, 1984, 166-169). A commercial product consisting of such an isolate diagnoses distinctly more sera as being HIV-2 positive than does the described standard isolate HTLV-$III_B$.

An ideal diagnostic or therapeutic product should contain at least one representative from the populations as significantly biologically distinguished from one another.

HIV-1 viruses in a multitude of highly polymorphic genetic mutants may cause different diseases such as ARC, LAS, AIDS and encephalopathies (ARC: AIDS-related complex, LAS: lymphadenopathy syndrome, AIDS acquired Immune deficiency syndrom). Cloned virus variants are distinguished in sequence and restriction pattern, even if they have been isolated at the same time, at the same place and even from the same patient (Rübsamen, H. et al., 1986). It could be shown that virus variants of the HIV-1 type are distinguished in some virus antigens up to about 15%. HIV-2's are even different in more than 40% of the aminoacids in some antigens, substitutions, insertions and deletions having been considered (Guyader, M. et al., 1987; Rabson, A.B. & Martin, M.A. "Cell" 40, 1985, 477-480).

The present invention provides a variant of the HIV-2 virus. The variant was isolated from a clinically asymptomatic patient. The virus isolate proved to be diagnostic agents, relative to DNA/RNA as well as relative to the virus antigens, for serologically and directly identifying infections by the type HIV-2 in the pre-AIDS and AIDS stages.

The virus isolate according to the invention comprises viruses and proviruses, the characteristics of which are identical to those of the disclosed restriction map and the sequence of the cloned partial regions (Figures 1-4). Moreover, the virus isolate comprises variants which are distinguished from the viruses and proviruses described above in that they are different in their nucleotide sequences from the above-described viruses only by up to 5%, and preferably by 2%, particularly preferred by 1%.

The virus variant according to the invention may cause lymphadenopathies (further designated as LAS/AIDS). Claimed according to the invention are also expression products of said virus variant, and more particularly antigens, preferably in accumulated or pure form, and processes for producing said expression

products in full or in parts or in combinations of the parts. The expression products are intended to include all polypeptides in glycosylated and or meristylated forms which have been coded on the positive or negative strand of the cloned RNA or DNA.

A further preferred embodiment consists of cloned DNA sequences capable of hybridizing with genomic RNA and DNA of the virus variant. Claimed according to the invention are stable gene probes containing such DNA sequences which are suitable for the detection of hybridization of those and other HIV variants or related viruses or DNA proviruses in samples to be investigated, more particularly biological or semi-synthetic samples.

A further preferred embodiment of the invention is comprised by virus variant the RNA/DNA of which or respective fragments will hybridize to the virus variants according to the invention under stringent conditions, more particularly c-DNA, genomic DNA, recombinant DNA, synthetic DNA or fragments thereof. These are understood to include variants or fragments which exhibit deletions and insertions in comparison to the virus variant according to the invention.

Stringent conditions of hybridization and washing are meant to be understood as those conditions which ensue by way of experiment or calculation if the melting point of the 100% homologous nucleic acid complexes in conditions of hybridization and washing will be fallen below by not more than 5 °C under the buffer conditions employed.

Also claimed according to the invention are cloned synthetic gene probes which may be derived from the above-described virus variants and can be augmented in vector systems in eukaryotes or prokaryotes. The described cloned DNA fragments are suitable for hybridization with complementary nucleic acids (DNA/RNA) for the purpose of diagnostic detection of the virus variants. The diagnostic tests according to the invention are carried out by using DNA or RNA probes. The probes are radioactive or have been labelled with fluorescent bio- or chemiluminescent groups or enzymes or are specifically detectable with enzymes via coupled reaction systems. The hybridizations may be effected in a homogeneous phase of a solution or in a heterogeneous phase with solid-immobilized nucleic acids, while the solid may be a membrane, particle, cell or tissue, so that the hybridization may also be effected in situ.

From the virus isolate claimed according to the invention, the corresponding DNA sequences (Figure 1) may be cloned in E. coli bacteria by establishing a genomic lambda-gene bank, starting from the DNA of the lymphocytes infected with the virus isolate. The desired clones are obtained by carrying out a plaque-screening with STLV-III sequences of the gag-pol range. In a more specifical way, there may be used as a probe a DNA derived from the published sequence HIV-2 ROD (Guyader, M. et al., "Nature" 326, 1987, 662-669), or a DNA probe derived from the partial sequences of the isolate HIV-2 D205 according to the invention.

The diagnostic method based on the use of the viruses claimed according to the invention comprises the following steps: Extraction of RNA or DNA from biological samples, possibly enzymatic processing by restriction enzymes, separation by gel electrophoresis and/or direct blot methods for nucleic acid-binding carriers, and subsequent hybridization with parts of the cloned fragments of the claimed viruses. Hybridizations may also be directly carried out in chemically treated cells or tissues. Therein the origin of the tissues or liquids is insiginificant.

Specifically, a process for the in vitro detection of antibodies against expression products of the viruses of the present invention is characterized in that the expression products or parts thereof of the viruses are detected by means of immunological methods. The process is characterized in that the expression products are proteins, peptides or parts thereof which have been coded within the meaning of an open reading frame on the DNA of the proviral partial sequences as characterized in claim 1 and are prepared by synthetic or biosynthetic processes.

The process is further characterized in that previously a definite amount or a combination of expression products or parts thereof are fixed on microtiter plates, whereupon subsequently biological samples, diluted or undiluted, are contacted with the coated microtiter plates and after incubation and sequential washing steps can be identified by means of a detecting reagent or of labelled anti-HIV antibodies.

Alternatively, filter strips and plastic strips or rods are used instead of microtiter plates, wherein the expression products of the viruses have been fixed at respective specific positions by isolated application of the different antigens.

The expression products or parts thereof can also be separated by gel electrophoresis and then transferred by blotting whereupon incubation with anti-HIV antibodies and the detection thereof are effected. Detection is effected on solid phase carriers to which the antigen determinants have been bonded, with the solid phase carrier consisting of particles.

Expression products can be virus antigens derived from in vitro-infected cells, said antigens being contacted with biological test materials as antigens bonded to fixed cells, and that the subsequent antibody

bonding can be determined with immunological detection reagents by means of an apparatus, for example with a cytofluorimeter, or visually.

The antigens can be determined by competitive ELISA. HIV-related nucleic acids (DNA and RNA) can be detected in biological samples, cells and in isolated form by using the nucleic acids according tc the present invention.

Expression products can be supplemented by materials which are related to other HIV variants, which, however, are distinguished in their biological properties from the materials of the isolates of the present invention.

For diagnostic and therapeutic goals the described DNA segments may also be employed for expressing coded antigens, parts thereof or combinations thereof with alien antigens. Therein the DNA segments under aimed control of regulation sequences are introduced into pro- or eukaryotic target cells, tissues or multiple-cell organisms to stimulate these to produce the accordingly coded antigens, parts thereof or combinations thereof with alien antigens. Antigens can be detected via the reaction with anti-HIV-2 antibodies, more particularly from the sera of the respective patients. Antigens having longer open reading frames (>50 amino acids) lend themselves as well those which are subject to splicing processes on the RNA level and are only thus composed to form the longer open reading frames.

According to the invention further claimed are polypeptides originating from the cloned virus variant according to the invention to detect such antigens in the material under investigation which contain similar antigen determinants and thereby do immunologically cross-react. This is particularly suitable for the diagnosis of AIDS and pre-AIDS of virus carriers or asymptomatic virus carriers or virus products, respectively, which are derived from blood. Also the serological detection of the antibodies directed against these antigenic polypeptides as expression products of the virus claimed according to the invention becomes possible by employing conventional systems such as ELISA. The immunogenic polypeptides may be used as protective polypeptides as vaccines to cause protection against AIDS infections.

The polypeptides according to the invention are understood to include fragments which are intentionally obtained by means of gene-technological methods, starting from longer open reading frames as well as those obtained by proteolytic enzymes in the production bacterial strains or in vitro by the use of proteases.

The virus isolates according to the invention and the products derived therefom may be combined with other isolates of the partial population HIV-2 in test systems, that is with those which are as far remote as possible in the described population level such as for example, the isolate HIV-2 ROD (Guyader, M. et al., 1987). Thereby it becomes possible sensitively to detect also populations of remote relationship in one test.

The virus variant according to the invention is highly different from the spectrum of the HIV-1 variants and have a closer molecular relationship to the HIV-2 virus described by Guyader, although they are distinguished therefrom to a significant extent (Figure 1). Also the biological properties are clearly distinguished from the described HIV-2 isolate. Thus, the variant according to the invention, for the effective in vitro replication, prefers cells which are derived from myeloidic lines. On the contrary, the virus poorly reproduces itself on lymphocytic lines.

A sample of the virus claimed according to the invention has been deposited in the form of its isolate at the European Collection of Animal Cell Cultures under the designation HIV D205 (V 87122304) according to the Budapest Treaty.

Figure 1 shows the restriction maps of the virus isolate according to the invention in comparison to known HIV sequences.

Figure 2 shows the partial nucleotide sequences of HIV-D205 (corresponding to clone HIV-2 A7.1 of Figure 2).

Figure 3 shows the sequence homology of HIV-2 D205,7 compared to the HIV/SIV group (gene level; nt/aa).

Figure 4 shows a nucleotide sequence comparison of HIV-2 D205 with HIV and SIV strains (in % homology).

Experimental results and characteristics of HIV-D205 are described in Kühnel, H. et al. (1989) Proc. Natl. Acad. Sci. 86, 4, 2383-2387.

The sequence of HIV-D205 shows a lot of so-called "open reading frames". Most of these reading frames can be related to in vivo expressed proteins/antigens by comparison of homologies to previously described HIV-viruses, by comparison of Western blots performed with HIV-D205 antigens derived from infected HUT78 or J937 cells and by probing with sera from the corresponding patients and reference sera.

Other open reading frames are not identified on the level of their expressed antigens defined by function or antibody staining on Western Blot. However, they can be expressed under some circumstances in vivo. Other reading frames, even short ones, can be expressed as well in a way difficult to predict solely on the basic of nucleic acid sequencing data because of splicing processes.

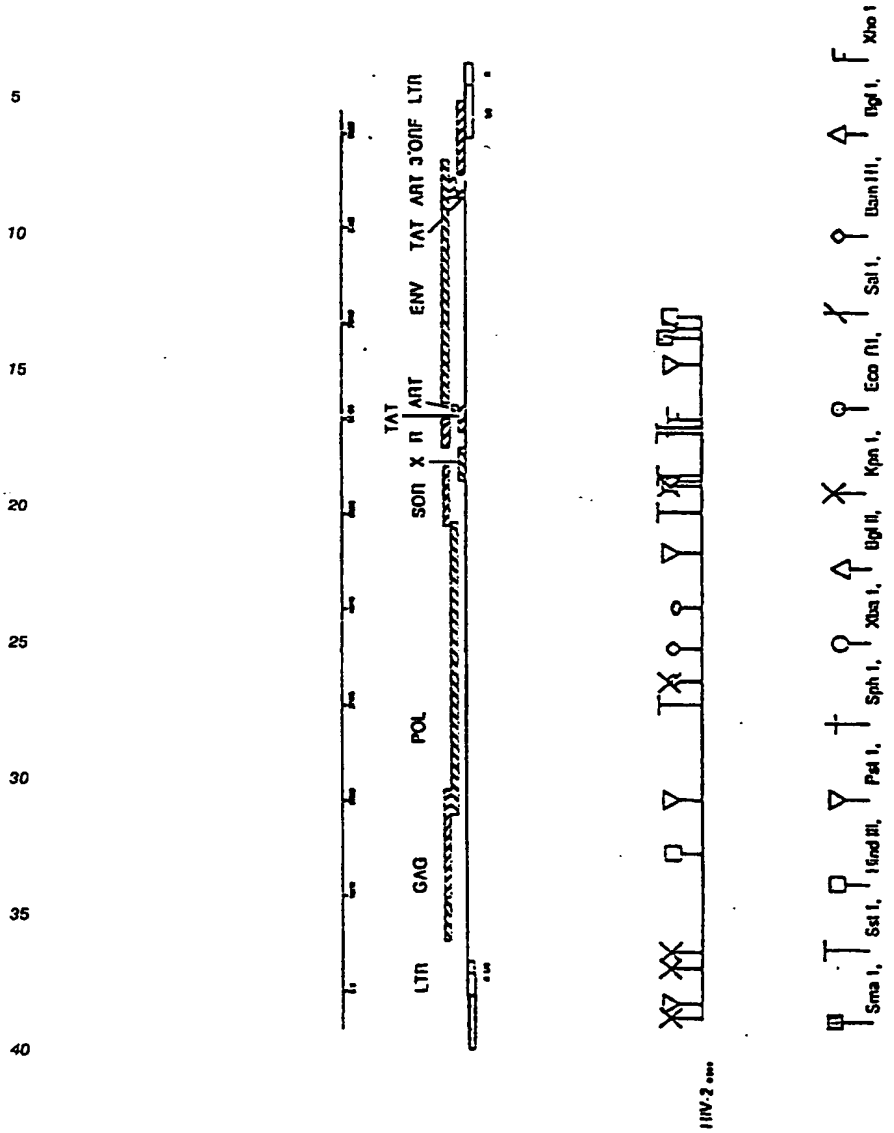
Antigenic determinants on expressed proteins as they are important for the biological function, for target antigens in diagnostics or for immunization are spread all over the expressed linear protein sequence. Parts of these sequences can have more general anticenic properties than others as can be shown by peptide screening/ mapping for antigenic sites. These sites can be expressed as single epitopes or as continuous polypeptide or in a version of in vitro or synthetically spliced antigens. Antigenicity of the expressed products can be demonstrated by antigen fixation and blotting in the Western Blot assay. Constructions for antigen expression in E. coli can be done by using conventional techniques using synthetic genes, restriction fragments from cloned viral genome segments, trimming products thereof by using exonuclease or DNase I or by using sequence specific synthetic primers defining the desired 5' and 3' end of the fragment to be expressed together with appropriate restriction sites. These restriction sites can easily be used for ligation into a panel of expression vectors of different organisms like those derived from PLc24 (Remault et al. 1981 Gene 15, 81-83) with multicloning sites (pEX).

The expressed antigens were shown to specifically react with patients' sera. The p27(24) from gag of HIV-D205 react very sensitively with both typical HIV-1 sera and typical HIV-2 sera (see Kühnel et al).

## Claims

1. A virus isolate HIV D205 (ECACC V 87122304).

2. DNA of the proviral partial sequences according to the following restriction endonuclease section-site characteristics, within the scope of the possible and conventional variation of errors, formed in establishing restriction maps.




3. cDNA and -fragments of the virus isolates according to claim 1.

4. Viral RNA and its fragments from virus isolates according to claim 1.

5. Recombinant DNA containing DNA pieces, starting from the virus isolates according to claim 1.

6. DNA or RNA of the virus isolates according to any one of the claims 1 to 4, wherein the DNA or RNA is present as hybride with complementary labelled DNA or RNA strands.

7. DNA according to any one of the claims 1 to 5, characterized in that it is complementary to viral DNA or parts thereof.

8. Nucleic acid strands in a modified or unmodified form which under stringent conditions hybridize with nucleic acids according to claims 2 to 7, and more specifically those nucleic acids which correspond to the highly variable regions of the HIV genom, more particularly in the range of the region coding the envelope protein.

9. Expression products of the virus isolates according to claim 1.

10. Expression products according to claim 1, characterized in that the proteins, peptides or fragments have been coded within the meaning of an open reading frame on the DNA according to claim 2.

11. A process for the in vitro detection of antibodies against expression products of the viruses according to claim 1, characterized in that the expression products or parts thereof of the viruses are detected by means of immunological methods.

12. The process according to claim 11, characterized in that the expression products are proteins, peptides or parts thereof which have been coded within the meaning of an open reading frame on the DNA according to claim 2 and are prepared by synthetic or biosynthetic processes.

13. The process according to claims 11 or 12, characterized in that previously a definite amount or a combination of expression products or parts thereof are fixed on microtiter plates, whereupon subsequently biological samples, diluted or undiluted, are contacted with the coated microtiter plates and after incubation and sequential washing steps can be identified by means of a detecting reagent or of labelled anti-HIV antibodies.

14. The process according to any one of claims 11 to 13, characterized in that filter strips and plastic strips or rods are used instead of microtiter plates, wherein the expression products of the viruses have been fixed at respective specific positions by isolated application of the different antigens.

15. The process according to claim 14, characterized in that the expression products or parts thereof are separated by gel electrophoresis and then transferred by blotting whereupon incubation with anti-HIV antibodies and the detection thereof are effected.

16. The process according to any one of claims 11 to 15, characterized in that the detection is effected on solid phase carriers to which the antigen determinants have been bonded. the solid phase carrier consisting of particles.

17. The process according to any one of claims 11 to 16, characterized in that the expression products are virus antigens derived from in vitro-infected cells, said anti-genes being contacted with biological test materials as antigens bonded to fixed cells, and that the subsequent antibody bonding can be determined with immunological detection reagents by means of an apparatus, for example with a cytofluorimeter, or visually.

18. The process according to any one of claims 11 to 17, characterized in that the antigens are determined by competitive ELISA.

19. A process for detecting HIV-related nucleic acids (DNA and RNA) in biological samples, cells and in isolated form by using the nucleic acids according to claims 2 to 7.

20. The process according to any one of claims 11 to 19, characterized in that the expression products are supplemented by materials which are related to other HIV variants, which, however, are distinguished in their biological properties from the materials of the isolates according to claim 1.

21. Immunogenic composition, containing expression products such as antigens, coded by the viruses of the virus isolates according to claim 1.

22. The immunogenic composition according to claim 21, characterized in that one antigen constitutes part of the total membrane antigen or is the total membrane antigen or a derivative thereof or a mixture of parts of the membrane antigens.

23. Antibodies, and more specifically monoclonal antibodies, against expression products of the virus isolates according to claim 1.

24. Cells which have been transformed with nucleic acids according to any one of claims 2 to 7.

25. Cells which have been infected with virus isolates according to claim 1.

Fig. 1

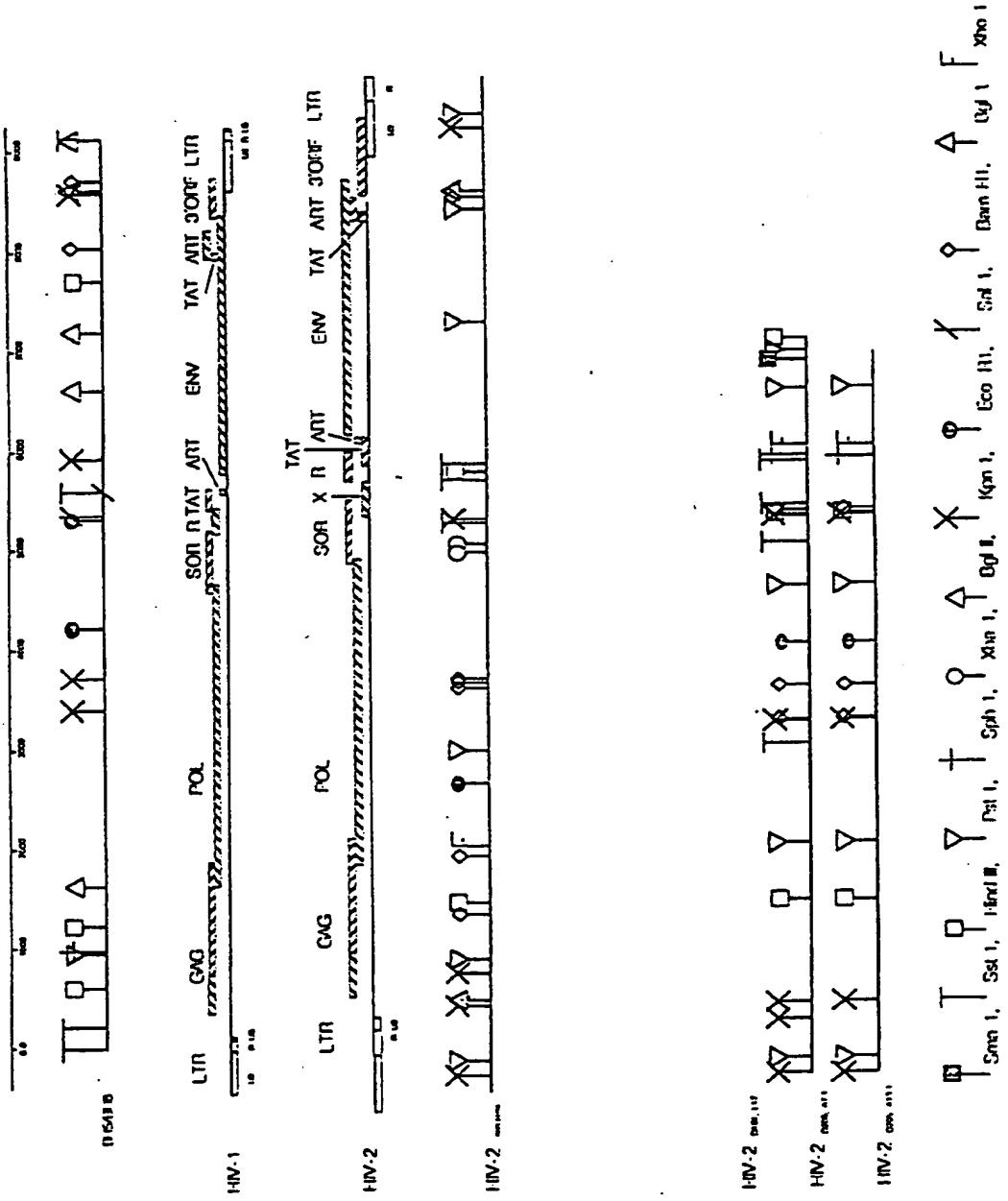

HIV-1
LTR
GAG
POL
ENV
TAT
ART
3'ORF
LTR
HIV-2
HIV-2
HIV-2
HIV-2
HIV-2
HIV-2
Sma I,
Sal I,
Hind III,
Pst I,
Sph I,
Xba I,
Bgl II,
Kpn I,
Eco RI,
Sst I,
Bam HI,
Cla I
Xho I

Fig. 2

Partial nucleotide sequences of HIV-D205
(corresponding to clone HIV-2 A7.1 of Fig. 2);

HIV-D205; corresponding to pos. 8942-9255 in HIV-2 ROD; homology 71.6 %

```
        10          20          30          40          50          60
TGGAAGGGAT GTATTATAGT GAGAGAAGAC ACAGAATATT AGACACATAT TTTGAGAATG

        70          80          90         100         110         120
AAGAAGGCAT TGTGTCTGGC TGGCAAAACT ATACTCATGG GCCAGGGATA AGGCATCCCA

       130         140         150         160         170         180
AATACTTTGG TTGGCTGTGG AAGCTGGTAC CAGTAGAGGT GCCAGCAGCG ACCCGAGAGG

       190         200         210         220         230         240
AGGAGGAAAC CCATTGCCTA ATGCACCCGG CACAGATCTC CTCATGGGAT GACATCCATG

       250         260         270         280         290         300
GGGAGACTCT TATCTGGCAG TTTGATTCCC TCCTGGCATA TGATTATGTG GCTTTCAATA

       310
GGTTTCCAGA AGAGTTT
```

HIV-D205, corresponding to position 718-2510 in HIV-2ROD; homology 78.6 %

```
        10          20          30          40          50          60
AAAAAATTCT TAAAGTCTTA GCTCCATTAG TACCAACAGG GTCAGAAAAT TTAAAAAGCC

        70          80          90         100         110         120
TTTTTAATAT CGTCTGCGTC ATTTTTTGCC TGCACGCAGA AGAGAAAGTG AAAGATACAG

       130         140         150         160         170         180
AGGAAGCAAA AAAGATAGCA CAGAGACATC TAGCGGCGGA CACAGAAAAA ATGCCAGCTA

       190         200         210         220         230         240
CAAATAAACC AACAGCACCA CCTAGCGGCG GAAATTATCC AGTGCAGCAA CTGGCTGGCA

       250         260         270         280         290         300
ACTACGTCCA CCTGCCGCTA AGCCCCCGAA CCTTAAATGC TTGGGTAAAG TTAGTAGAAG

       310         320         330         340         350         360
AAAAGAAGTT CGGGGCAGAA GTAGTACCAG GATTTCAGGC ACTATCAGAA GGATGCACCC

       370         380         390         400         410         420
CTTATGATAT AAATCAGATG CTAAATTGTG TAGGAGAACA TCAGGCAGCC ATGCAAATTA

       430         440         450         460         470         480
TTAGAGAAAT AATCAATGAG GAAGCAGCAG ACTGGGACCA GCAACACCCG TCACCAGGCC
```

Fig. 2

```
       490         500         510         520         530         540
CAATGCCGGC AGGACAACTT AGGGACCCAA GAGGGTCAGA TATAGCAGGA ACCACCAGCA

       550         560         570         580         590         600
CAGTAGAGGA ACAGATACAG TGGATGTACA GGGCCCAAAA TCCTGTCCCA GTGGGAAACA

       610         620         630         640         650         660
TTTATAGAAG ATGGATTCAA TTAGGATTGC AGAAATGTGT CCGAATGTAC AATCCTACCA

       670         680         690         700         710         720
ACATATTAGA CATAAAGCAG GGACCAAAGG AGCCCTTCCA AAGCTATGTA GATAGATTCT

       730         740         750         760         770         780
ACAAAAGCTT ACGGGCAGAA CAAACAGACC CAGCAGTGAA AAATTGGATG ACACAAACAC

       790         800         810         820         830         840
TGCTGATTCA GAATGCTAAC CCAGATTGCA AGTTAGTGCT TAAGGGCTTG GGAATGAATC

       850         860         870         880         890         900
CCACCTTAGA GGAAATGCTA ACGGCCTGCC AAGGGATAGG AGGCCCAGGG CAGAAGGCAA

       910         920         930         940         950         960
GGCTAATGGC CGAAGCCTTA AAAGAGGCCC TAACACCTGC ACCCATACCG TTTGCTGCCG

       970         980         990        1000        1010        1020
TTCAACAAAA AGCAGGGAAG AGAGGGACAG TGACATGCTG GAACTGTGGC AAACAGGGAC

      1030        1040        1050        1060        1070        1080
ACACAGCCAG GCAATGCAGG GCCCCTAGAA GACAGGGATG CTGGAAATGT GGAAAAACAG

      1090        1100        1110        1120        1130        1140
GACACATCAT GTCAAAATGC CCAGAAAGAC AGGCGGGTTT TTTAGGGTTA GGACCCTGGG

      1150        1160        1170        1180        1190        1200
GAAAGAAGCC TCGCAACTTC CCCATGACCC AAGTGCCTCA GGGAGTGACA CCATCTGCAC

      1210        1220        1230        1240        1250        1260
CCCCGATGAA CCCAGCAGAG GGCATGACAC CTCGGGGGGC GACACCATCT GCGCCCCCTG

      1270        1280        1290        1300        1310        1320
CAGATCCAGC AGTGGAGATG CTGAAAAGTT ACATGCAGAT GGGGAGACAA CAGAGAGAGA

      1330        1340        1350        1360        1370        1380
GCCGAGAGAG ACCCTACAAG GAGGTGACAG AGGATTTGCT GCACCTCAAT TCTCTCTTTG

      1390        1400        1410        1420        1430        1440
GAGAAGACCA GTAGTCAAAG CATGTATCGA GGGTCAGTCA GTAGAAGTAT TACTAGACAC

      1450        1460        1470        1480        1490        1500
AGGAGTTGAC GACTCAATAG TAGCAGGGAT AGAATTAGGT AGCAATTACA CCCCAAAAAT

      1510        1520        1530        1540        1550        1560
AGTAGGAGGG ATAGGAGGGT TCATAAATAC CAAAGAATAC AAAGATGTAG AAATAGAAGT

      1570        1580        1590        1600        1610        1620
AGTGGGAAAA AGAGTAAGGG CAACTATAAT GACAGGAGAT ACCCCAATAA ACATTTTTGG
```

Fig. 2

```
      1630        1640        1650        1660        1670        1680
CAGAAATATT TTAAATACCT TGGGCATGAC TTTAAATTTC CCAGTGGCAA AGGTAGAACC

      1690        1700        1710        1720        1730        1740
AGTAAAAGTT GAGTTAAAAC CTGGAAAAGA TGGGCCAAAG ATCAGACAAT GGCCTCTATC

      1750        1760        1770        1780        1790
CAGGGAAAAG ATACTAGCCC TCAAAGAAAT CTGTGAAAAA ATGGAAAAGG
```

HIV-D205, corresponding to position 2877-7293 in HIV-2$_{ROD}$; homology 75.1 %.

```
        10          20          30          40          50          60
AGGTATTAGA TCCTTTTAGA AAGGCCAACA GCGATGTCAT TATAATTCAG TACATGGATG

        70          80          90         100         110         120
ACATCCTTAT AGCAAGTGAC AGAAGTGATC TGGAGCACGA CAGGGTAGTG TCCCAACTAA

       130         140         150         160         170         180
AAGAGTTATT AAATGACATG GGATTCTCTA CCCCAGAAGA AAAGTTCCAA AAAGACCCTC

       190         200         210         220         230         240
CGTTCAAATG GATGGGTTAT GAGCTCTGGC CAAAAAAGTG GAAACTGCAA AAAATACAAC

       250         260         270         280         290         300
TGCCAGAAAA AGAAGTTTGG ACAGTGAATG CAATTCAAAA ACTGGTAGGA GTATTAAACT

       310         320         330         340         350         360
GGGCAGCTCA ACTCTTTCCT GGAATTAAGA CAAGGCACAT ATGCAAACTA ATTAGGGGAA

       370         380         390         400         410         420
AGATGACCCT AACAGAAGAA GTACAGTGGA CAGAACTAGC AGAAGCAGAG CTACAGGAGA

       430         440         450         460         470         480
ATAAAATCAT CTTAGAACAG GAACAAGAAG GATCCTACTA CAAGGAAAGG GTACCGCTAG

       490         500         510         520         530         540
AAGCAACAGT ACAGAAAAAC CTAGCAAATC AGTGGACATA CAAAATTCAT CAGGGAAATA

       550         560         570         580         590         600
AAGTCCTAAA AGTAGGAAAA TATGCAAAGG TTAAAAACAC GCACACCAAC GGGGTAAGAC

       610         620         630         640         650         660
TACTGGCACA TGTAGTTCAG AAAATAGGCA AAGAAGCCCT AGTCATCTGG GGAGAGATAC

       670         680         690         700         710         720
CAGTGTTCCA TCTGCCAGTA GAAAGAGAGA CATGGGACCA GTGGTGGACA GATTACTGGC

       730         740         750         760         770         780
AAGTAACCTG GATCCCAGAG TGGGACTTTG TCTCGACCCC ACCATTAATA AGACTAGCCT

       790         800         810         820         830         840
ACAACCTAGT CAAAGACCCC CTAGAAGGGA GAGAAACCTA CTACACAGAT GGGTCCTGCA
```

Fig. 2

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      850         860         870         880         890         900
ATAGAACCTC AAAGGAAGGA AAAGCAGGAT ATGTCACTGA CAGGGGAAAA GATAAGGTTA

      910         920         930         940         950         960
AAGTGTTAGA ACAGACAACA AACCAACAAG CAGAACTTGA AGCATTTGCA TTAGCATTAA

      970         980         990        1000        1010        1020
CAGACTCAGA ACCACAAGTT AACATCATAG TAGATTCACA ATATGTCATG GGAATAATAG

     1030        1040        1050        1060        1070        1080
CTGCACAGCC AACAGAAACA GAATCACCAA TAGTAGCAAA AATAATTGAA GAAATGATCA

     1090        1100        1110        1120        1130        1140
AAAAAGAGGC AGTATATGTA GGATGGGTAC CAGCTCACAA GGGACTGGGT GGTAATCAGG

     1150        1160        1170        1180        1190        1200
AAGTAGACCA CCTAGTAAGT CAAGGAATCA GACAGGTCTT GTTCCTAGAA AAAATAGAAC

     1210        1220        1230        1240        1250        1260
CAGCCCAGGA AGAGCATGAA AAATATCATG GCAATGTAAA AGAACTGGTC CATAAATTCG

     1270        1280        1290        1300        1310        1320
GAATTCCACA ATTAGTGGCA AAACAGATAG TAAATTCCTG TGATAAATGC CAACAAAAAG

     1330        1340        1350        1360        1370        1380
GGGAAGCTAT TCATGGACAG GTAAATGCAG ACCTAGGGAC ATGGCAGATG GACTGTACAC

     1390        1400        1410        1420        1430        1440
ATTTAGAAGG AAAAATTATA ATAGTGGCAG TCCATGTAGC CAGTGGGTTT ATAGAAGCAG

     1450        1460        1470        1480        1490        1500
AGGTAATACC CCAAGAGACA GGAAGACAGA CAGCTCTCTT CCTACTAAAG TTGGCCAGCA

     1510        1520        1530        1540        1550        1560
GATGGCCTAT CACACACCTA CACACAGACA ACGGTGCCAA CTTCACCTCA CCAAGTGTAA

     1570        1580        1590        1600        1610        1620
AGATGGTAGC CTGGTGGGTA GGAATAGAAC AAACTTTTGG AGTACCCTAT AACCCACAAA

     1630        1640        1650        1660        1670        1680
GTCAAGGAGT AGTGGAAGCA ATGAACCATC ACCTGAAAAA TCAAATAGAC AGACTCAGAG

     1690        1700        1710        1720        1730        1740
ACCAAGCAGT ATCAATAGAG ACAGTTGTAC TAATGGCAAC TCACTGCATG AATTTTAAAA

     1750        1760        1770        1780        1790        1800
GAAGGGGAGG AATAGGGGAT ATGACCCCTG CAGAAAGACT AGTTAACATG ATAACCACAG

     1810        1820        1830        1840        1850        1860
AGCAAGAAAT ACAGTTCTTC CAAGCAAAAA ATTTAAAATT TCAAAATTTC CAGGTCTATT

     1870        1880        1890        1900        1910        1920
ACAGAGAAGG CAGAGATCAA CTCTGGAAGG GACCTGGTGA ACTATTGTGG AAAGGGGAAG

     1930        1940        1950        1960        1970        1980
GAGCAGTCAT CATAAAGGTA GGGACAGAAA TCAAAGTAGT ACCCAGGAGA AAAGCAAAAA
```

Fig. 2

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      1990        2000        2010        2020        2030        2040
TTATAAGGCA CTATGGAGGA GGAAAAGGAT TGGATTGTAG TGCCGACATG GAGGATACCA

      2050        2060        2070        2080        2090        2100
GGCAGGCTAG AGAGATGGCA CAGTCTGATT AAGTATCTTA AGTATAGAAC AGGAGAGTTG

      2110        2120        2130        2140        2150        2160
CAACAGGTCT CTTATGTCCC TCACCACAAG GTAGGATGGG CTTGGTGGAC TTGCAGTAGA

      2170        2180        2190        2200        2210        2220
ATAATATTTC CCCTAAACAA AGGAGCATGG CTAGAAGTCC AAGGATATTG GAACCTAACC

      2230        2240        2250        2260        2270        2280
CCAGAAAGGG GATTCTTGAG CTCCTATGCT GTAAGACTAA CATGGTATGA GAGGAACTTT

      2290        2300        2310        2320        2330        2340
TATACAGATG TAACACCTGA TGTGGCAGAC CAGCTACTGC ATGGGTCTTA TTTCTCTTGC

      2350        2360        2370        2380        2390        2400
TTTTCAGCCA ATGAAGTAAG GAGAGCCATC AGGGGAGAAA AGATATTGTC CTACTGCAAC

      2410        2420        2430        2440        2450        2460
TATCCATCAG CTCACGAAGG GCAGGTACCA AGCTTACAGT TTCTAGCCCT AAGGGTCGTA

      2470        2480        2490        2500        2510        2520
CAGGAAGGAA AAAATGGATC CCAGGGAGAG AGTGCCACCA GGAAACAGCG ACGAAGAAAC

      2530        2540        2550        2560        2570        2580
AGTAGGAGAA GCATTCGCTT GGCTAGAAAG AACAATAACA GAGCTCAACA GGGTAGCGGT

      2590        2600        2610        2620        2630        2640
CAACCATTTG CCCCGAGAAC TTATTTTCCA GGTCTGGCAG AGGTCTTGGG CATACTGGCG

      2650        2660        2670        2680        2690        2700
TGAGGAACAG GGCATGTCAA TTAGCTATAC CAAATATAGA TACTTGTTGC TAATGCAGAA

      2710        2720        2730        2740        2750        2760
AGCAATGTTT GTGCACTATA CAAAGGGCTG TAGGTGCCTG CAGGAGGGCC ATGGGCCAGG

      2770        2780        2790        2800        2810        2820
GGGATNGAGA TCAGGACCTC CTCCTCCTCC TCCCCCAGGC CTGGCCTAAT GGCAGAAGCA

      2830        2840        2850        2860        2870        2880
GCCCCAGAGA TCCCTCCAGA GAACGAGAAC CCACAAAGAG AACCGTGGGA AGAGTGGATA

      2890        2900        2910        2920        2930        2940
GGGGAGATCC TGGAGGAAAT AAAGCAAGAA GCCTTAAAGC ATTTTGATCC TCGCTTGCTA

      2950        2960        2970        2980        2990        3000
ACTGCGCTTG GTAACTTTAT CTACAGTAGG CATGGAGATA CCCTTGCAGG AGCAGGAGAG

      3010        3020        3030        3040        3050        3060
CTCATTAAAA TCCTCCAACG AGCNCTCTTC CTCCACTTCA GAGCCGGTTG TCAACACTCA

      3070        3080        3090        3100        3110        3120
AGGATTGGAC AATCAGGGGG AGGAAATCCT CTCTCAACTA TACCGCCCCC TTAAGGCATG
```

Fig. 2

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      3130        3140        3150        3160        3170        3180
CGATAATACA TGCTACTGTA AGAAATGCTG CTACCATTGC CAGCTTTGTT TTCTTAAAAA

      3190        3200        3210        3220        3230        3240
GGGTCTTGGG ATATGTTATG ACCGCTCGAG AAGGAGATCT GCAAAAAGAG CTAAGACTAC

      3250        3260        3270        3280        3290        3300
TGCACCTTCT GCACCAGACA AGTGAGTATG GCATATTTTA GCAGCCGCCT GCCTATTGCG

      3310        3320        3330        3340        3350        3360
CTCCTGCTTA TAGGTATCAG TGGGTTTGTA TGTAAACAAT ATGTTACTGT CTTCTATGGC

      3370        3380        3390        3400        3410        3420
ATACCCGCAT GGAGGAACGC AACAGTTCCC CTCATTTGTG CAACCACAAA CAGAGACACC

      3430        3440        3450        3460        3470        3480
TGGGGAACTG TACAGTGTCT CCCAGACAAT GGTGACTACA CTGAGATCAG GCTAAACATA

      3490        3500        3510        3520        3530        3540
ACAGAGGCTT TTGATGCATG GGATAATACA GTGACACAAC AGGCAGTAGA TGATGTGTGG

      3550        3560        3570        3580        3590        3600
AGACTCTTTG AAACCTCCAT AAAACCATGT GTCAAACTAA CCCCACTGTG TGTGGCAATG

      3610        3620        3630        3640        3650        3660
AACTGTAGTA AAACCGAAAC AAACCCAGGG AATGCCAGTA GTACTACCAC CACTAAGCCT

      3670        3680        3690        3700        3710        3720
ACTACCACCT CTCGTGGGCT GAAAACGATT AACGAAACAG ACCCATGCAT AAAAAATGAC

      3730        3740        3750        3760        3770        3780
AGCTGCACAG GACTAGGAGA AGAGGAAATA ATGCAATGTA ATTTTAGTAT GACGGGACTA

      3790        3800        3810        3820        3830        3840
AGAAGAGATG AGCTAAAACA ATATAAAGAC ACCTGGTACT CAGAAGATTT AGAGTGTAAT

      3850        3860        3870        3880        3890        3900
AATACCAGGA AGTAATACCA GCAGTGCTAT ATAAGAACCT GCAACACAAC AATTATCCAA

      3910        3920        3930        3940        3950        3960
GAGTCATGTG ACAAACATTA TTGGGACAGC TTAAGGTTTA GGTATTGTGC TCCCCCGGGG

      3970        3980        3990        4000        4010        4020
TTTTTTCTAC TAAGATGTAA TGATACCAAC TATTCAGGCT TCATGCCCAA CTGCAGTAAG

      4030        4040        4050        4060        4070        4080
GTAGTAGCGT CCTCCTGCAC AAGAATGATG GAAACACAGT CCTCTACATG GTTTGGCTTC

      4090        4100        4110        4120        4130        4140
AATGGTACAA GGGCAGAGAA CAGGACATAT ATATATTGGC ATGAAAAAGA CAATAGGACC

      4150        4160        4170        4180        4190        4200
ATCATAAGCT TAAATACATA CTATAATTTG TCAATACACT GTAAGAGGCC AGGAAACAAG

      4210        4220        4230        4240        4250        4260
ACGGTTGTAC CAATAAGAAC CGTGTCAGGA CTACTTTTCC ATTCACAGCC TATCAATAAG
```

Fig. 2

```
      4270        4280        4290        4300        4310        4320
AGACCCAGAC AAGCTTGGTG CTGGTTTAAG GGAAACTGGA CAGAAGCCAT AAAGGAGGTG

      4330        4340        4350        4360        4370        4380
AAAAGGACCA TCATAAAACA TCCCAGGTAT AAAGGAGGTG CAAAAAATAT CACAAGCGTA

      4390        4400        4410
AAGTTAGTAT CAGAACATGG AAAAGGTTCA GATC
```

Fig. 3

Sequence homology of HIV-$2_{D205,7}$ compared to the HIV/SIV group (gene level; nt / aa)

| | HIV-$2_{D205,7}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| gene | position | HIV-$2_{ROD}$ | HIV-$2_{NIHZ}$ | HIV-$2_{D194}$ | $SIV_{MAC}$ | $SIV_{AGM}$ | HIV-$1_{BRU}$ |
| gag | 720-1026 | 80.5 / 85.6 | | | | | |
| gag | 1860-2114 | 83.1 / 77.6 | | | | | |
| pol | 1859-2510 | 80.2 / 72.5 | | | | | |
| pol | 2877-4948 | 78.3 / 83.5 | | | | | |
| protease | 2084-2381 | 84.0 / 81.0 | 83.0 / 84.8 | 84.8 / 86.8 | 76.3 / 83.8 | 57.8 / 47.1 | 60.4 / 48.5 |
| vif | 4869-5516 | 72.0 / 68.5 | 70.9 / 67.9 | 72.4 / 66.5 | 71.8 / 60.6 | 53.8 / 34.7 | 47.9 / 33.0 |
| vpx | 5344-5682 | 76.1 / 74.1 | 73.5 / 68.1 | 74.6 / 77.9 | 75.2 / 77.0 | 50.8 / 34.7 | |
| vpr | 5682-5999 | 78.8 / 69.8 | 77.7 / 69.8 | 74.2 / 59.4 | 78.3 / 76.4 | | 51.9 / 47.3 |
| tatex1 | 5845-6140 | 78.4 / 66.3 | 79.1 / 68.4 | 74.7 / 63.3 | 81.1 / 66.3 | 33.1 / 38.1 | 33.6 / 34.0 |
| revex1 | 6071-6140 | 67.1 / 61.9 | 68.6 / 60.9 | 67.1 / 52.2 | 70.3 / 60.9 | 45.5 / 28.6 | 38.2 / 40.4 |
| nef | 8557-9255 | 72.1 / 69.5 | | | | | |
| env | 6147-7293 | 70.0 / 67.0 | | | | | |



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Fig. 4

Nucleotide sequence comparison of HIV-$2_{D205}$ with HIV and SIV strains (in % homology)

| HIV-$2_{D205}$ position | HIV-$2_{ROD}$ | HIV-$2_{NIHZ}$ | HIV-$2_{D194}$ | $SIV_{MAC}$ | $SIV_{AGM}$ | HIV-$1_{BRU}$ |
|---|---|---|---|---|---|---|
| 8942-9255 | 71.6 | 77.0 | 68.8 | 66.4 | 56.3 | 54.7 |
| 718-1825 | 80.5 | 80.8 | 80.3 | 79.1 | 65.1 | 63.8 |
| 1859-2510 | 80.2 | 74.6 | 75.0 | 70.8 | 55.6 | 56.9 |
| 2877-7293 | 75.1 | 74.8 | 75.4 | 74.0 | 58.0 | 54.6 |
| Total | 75.9 | 75.9 | 75.9 | 75.0 | 58.9 | 56.4 |



EP 0 655 501 A1

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# EUROPEAN SEARCH REPORT

Application Number
EP 95 10 0149

## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int.Cl.6) |
|---|---|---|---|
| A | SCIENCE.,<br>vol.240, no.4858, 10 June 1988, LANCASTER, PA US<br>pages 1522 - 1525<br>EVANS ET AL. 'Characterization of a nonpathogenic HIV-2 strain with unusual effects on CD4 expression'<br>* table 1 *<br>--- | 1-25 | C12N15/49<br>C12N7/00<br>C07K14/155<br>G01N33/569<br>A61K39/21<br>A61K39/395<br>C12N5/10 |
| A | AIDS RESEARCH AND HUMAN RETROVIRUSES,<br>vol.3, no.1, January 1987<br>pages 3 - 10<br>ALBERT ET AL. 'A new human retrovirus isolate of West African origin (SBL-6669) and its rlationship to HTLV-IV, LAV-II and HTLV-IIIB'<br>* page 4, last paragraph *<br>--- | 1-25 | |
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| P,X | PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA.,<br>vol.86, April 1989, WASHINGTON US<br>pages 2338 - 2387<br>KUHNEL ET AL. 'Molecular cloning of two West African human immunodeficiency virus type 2 isolates that replicate well inmacrophages'<br>* the whole document *<br>----- | 1-25 | |

The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| THE HAGUE | 17 February 1995 | Cupido, M |

CATEGORY OF CITED DOCUMENTS

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